IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Anna Helgadottir

Application No.: 10/829,674

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For: SUSCEPTIBILITY GENE FOR MYOCARDIAL INFARCTION AND STROKE

Confirmation No.: 6838

Art Unit: 1634

Examiner: J.A. Goldberg

DECLARATION OF ANNA HELGADOTTIR, M.D. UNDER 37 C.F.R. § 1.132

I, Anna Helgadottir, M.D., hereby declare as follows that:

1. I am a medical doctorate from The University of Iceland. I have been working at deCODE genetics, ehf. ("deCODE') as a project leader for the Myocardial Infarction Genetics group for 9 years. My current title at deCODE is Project Leader.

2. I am a named co-inventor of the above-identified U.S. patent application, which is assigned to deCODE. I am familiar with the contents and pending claims of this patent application and the examination report (Office Action) from the U.S. Patent and Trademark Office dated July 21, 2006. I am providing this declaration to make available to the Examiner additional data that may be relevant to issues raised in the Office Action and patentability of the claims. I collaborated or participated in the following procedures described herein.

3. In Section 5 of the Office Action (Scope of Enablement), the Examiner cited references that are asserted to demonstrate the unpredictability of associating genetic variations with relative risk for disease. The Examiner stated that genetic studies are "irreproducible" and many of the findings only demonstrate modest association with risk.

4. Many genetic association studies performed to date have suffered from study heterogeneity, *i.e.* the results from one study may be only poorly, if at all, replicated in the next. There are many

reasons for such lack of replication, including population stratification, lack of power, genetic heterogeneity, etc. Therefore, if an association is observed in several studies, it is likely to represent a significant finding. However, lack of replication of an initial finding in one, or even several, replication studies does not necessarily mean that the initial finding is discredited.

5. To demonstrate the asserted unpredictability in the art, the Examiner cited Meschia *et al.* (*Ann. Neurology* 58: 351-361, 2005), which reports that in a study of North American siblings the authors failed to detect a significant linkage between variants of ALOX5AP (FLAP) and ischemic stroke. Meschia *et al.* is primarily a linkage study rather than an association study similar to the studies described in the specification. Observed linkage signals depend strongly on the frequency and risk of the underlying genetic variants. Thus, the lack of linkage to the FLAP and PDE4D regions in Meschi *et al.* does not by any means undermine the significant linkage data reported by Helgadottir *et al.* (*Nature Genet.* 36:233-239, 2004; attached as Exhibit A) and Gretarsdottir *et al.* (*Am J Hum Genet*, 70:593-603, 2002; attached as Exhibit B). In addition, it should be stressed that lack of linkage signal does not indicate lack of association to genetic variants in the region. Thus, the study by Meschi *et al.* does not undermine the association results provided in the specification and herein.

6. Moreover, the present application describes associations (not linkages) of specific marker combinations (i.e. markers, haplotypes) with MI and stroke. It should be noted that Meschi *et al.*only show association results for single markers and not haplotype association results. Our findings have shown that the analysis of *haplotypes* leads to significant association to MI and stroke. Therefore, the association results presented in Meschi *et al.* realting to individual markers do not refute the data presented in the specification and herein.

7. The Examiner also cited two articles (Hirschhorn *et al. Gen. Med.* 45:45-61, 2002 and Ioannidis *et al. Nat. Genetics* 29: 306-309, 2001) that revealed the results of genetic association studies. Hirschhorn *et al.* reviewed 166 genetic associations to determine whether subsequent studies on the same polymorphism and disease also reached statistical significance. In their analysis, only 6 of the associations have been consistently replicated, however, overall 97 out of 166 associations were observed in one or more studies. Hirschhorn *et al.* suggests solutions that could remedy the observed "irreproducibility." This article is evidence that those of skill in the art at the time of filing

understood what is needed to properly carry out genetic association studies. For example, one of the possible solutions provided in Hirschhorn *et al.* is to use ethnic matching of cases and controls, and to analyze multiple case-controlled populations to increase power and to minimize the effect of population stratification in single studies. The association of FLAP polymorphisms and haplotypes with MI and stroke described in the specification were carried out in multiple populations (Icelandic, British, Scottish and North American). To address false positive association results due to population stratification, each cohort was analyzed separately, and for each US cohort European Americans (Caucasians) were analyzed separately from other ethnic groups.

8. Ioannidis *et al.* compared the analysis of 370 genetic studies. The results of this analysis cautioned that a strong association in the first study typically becomes gradually less prominent as more data accumulates. However, the analysis in Ioannidis *et al.* also revealed that in some studies, a first analysis did not find a statistically significant difference but with the accumulation of further data, the genetic association becomes formally statistically significant (see page 307, left column). Ioannidis *et al.* summarizes their analysis by stating that genetic association studies require cautious replication and that estimates may be inflated if only based on a single study with an impressive result. Ioannidis *et al.* concludes that a systematic meta-analytic approach may assist in estimating population-wide effects of genetic risk factors for human disease. The association of polymorphisms and haplotypes in the FLAP nucleic acid with risk for MI and stroke of the present invention have been analyzed in several populations and are not the result of a single small study. The data provided in the specification revealed a significant association of FLAP haplotypes with MI and stroke in an Icelandic cohort (see pages 83-85, pages 86-87 and pages 88-90 (Tables 4, 5, 7 and 9) of the specification), in a British and Scottish cohort (See paragraphs 10-13 and Table 1 of this declaration and Helgadottir *et al. Am J Hum Genet* 76:505-509, 2005; attached as Exhibit C) and North American cohorts (see paragraphs 10-13 and Table 1 of this declaration). Therefore, while Ioannidis *et al.* provide evidence that genetic studies may be unpredictable, a better estimate of the effects of the genetic variant may be obtained if the association studies are repeated with several large populations and a meta-analysis is performed.

9. The association of HapA to MI and stroke in the Icelandic population, as described at pages 83-90 of the specification, was observed in a British MI cohort, although the higher frequency of HapA in cases as compared with controls was not statistically significant (pages 91-92 of the

specification; Helgadottir *et al. Nat Genet* 36:233-239, 2004, p. 235; Exhibit A, and Table 1). The HapB haplotype within the FLAP gene was, however, found to be significantly associated with MI in the British population. A subsequent study replicated the association to stroke in a Scottish population, as disclosed by Helgadottir *et al.* (*Am J Hum Genet* 76:505-509, 2005; Exhibit C). These replication studies therefore provide further evidence for the involvement of the FLAP gene in the pathogenesis of MI and stroke.

10. Furthermore, we have performed additional replication studies on several MI cohorts from the United States, the United Kingdom and Iceland (Table 1). In these North American cohorts, and in a replication cohort from Iceland, the association of the FLAP haplotype HapA to MI replicates in the overall sample.

11. The case-control cohorts have been described previously (Dowling *et al.*, *J Thromb Haemost* 1:80-87, 2003, Exhibit D; Helgadottir *et al.*, *Nature Genet.* 36:233-239, 2004, Exhibit A), except for the Durham, NC cohort. In brief, the study participants from Philadelphia were enrolled at the University of Pennsylvania Medical Center through the PENN CATH study program, which studies the association of biochemical and genetic factors with coronary artery disease (CAD) in subjects undergoing cardiac catheterization. The study participants from Cleveland were enrolled at the Cleveland Clinic Heart Center through the Genebank program, which is a registry of data in conjunction with biological samples for individuals undergoing coronary catheterization. The case-control cohort from Atlanta was enrolled at the Emory University Hospital, the Emory Clinic and Grady Memorial Hospitals through its Emory Genebank study, the Clinical Registry in Neurology (CRIN), and the Genetic Attributes and Thrombosis Epidemiology (GATE) study. The Emory Genebank study analyzed the association of biochemical and genetic factors with CAD in subjects undergoing cardiac catheterization. The study participants from Durham, NC were enrolled at the Duke University Medical Center through the CATHGEN program, which is a registry of data in conjunction with biological samples for individuals undergoing coronary catheterization. MI patients from Iceland were recruited from a registry that includes all MIs diagnosed before the age of 75 in Iceland from 1981-2004, following WHO-MONICA criteria for acute MI. The controls used for this study were recruited through various genetic programs at deCODE. The cohort from UK was recruited from Sheffield and Leicester. All patients satisfied the WHO-MONICA criteria for acute MI. Controls were recruited from the general population of the same geographical region as the

patients. The study was approved by relevant Institutional Review Boards and all subjects provided written informed consent. Ethnicity was determined by self-reporting only in this study.

Table 1

Replication of the association of HapA to MI in cohorts from the United States, United Kingdom and Iceland.

Cohort	*P* value	RR	MI	Controls
European Americans				
Philadelphia (725/519)	0.748	0.93	0.159	0.170
Cleveland (648/743)	0.057	1.20	0.162	0.139
Atlanta (713/1434)	0.019	1.22	0.171	0.145
Durham, NC (830/489)	0.187	1.11	0.153	0.139
All MI US .coh adj (2916/3185)	0.021	**1.12 (1.00,1.25)**	0.161	0.147
United Kingdom (750/726)	0.211	1.09	0.169	0.157
All MI US+UK coh adj (3666/3911)	0.015	**1.11 (1.01,1.23)**	0.163	0.149
Iceland[a] rel adj (1067/14196)	0.037	**1.14**	0.142	0.126
All Caucasians				
Iceland[a] + US + UK (4733/18107)*	0.003	**1.12 (1.03, 122)**		
Physicians' Health Study (341/600)**	0.058	1.23	0.172	0.145

[a]The cohort from Iceland is independent from the initial discovery cohort, *i.e.* all patients and controls that were used in the association study, described in Helgadottir *et al.*, *Nature Genet.* 36:233-239 (2004), have been excluded.

*P-value adjusted for cohort and relatedness within the Icelandic cohort

** Zee et. al., Stroke 37, 2007-2011 (2006). P-value recalculated with a chi-squared test with one degree of freedom

12. Table 1 provides the results for the association analysis of HapA of the FLAP gene for each North American cohort, the cohort from the United Kingdom, and a replication cohort from Iceland (excluding the discovery cohort presented in Helgadottir *et al.*, *Nature Genet.* 36:233-239, 2004;

attached as Exhibit A) with the corresponding number of individuals genotyped (patients/controls), the one-sided *P* values, the relative risk (RR), and haplotype frequencies in MI patients and controls. HapA is defined by the following SNPs: SG13S25, SG13S114, SG13S89, and SG13S32, with alleles G, T, G, and A, respectively. A metaanalysis of the results for the cohorts from the United States, the cohort from United Kingdom and the replication cohort from Iceland are shown with adjustment for cohort-dependent haplotype frequencies (coh. adj.) and for relatedness of the patients within the Icelandic cohort (rel adj). It should be noted that this study includes the analysis of close to 5,000 cases and over 18,000 controls. As shown in Table 1, the RR was similar for all the cohorts analyzed, and the RR was greater than 1 for six out of the seven populations with an overall RR of 1.12. In addition, the *P* value decreased as the additional cohort data was included in the analysis and the data had a collective *P* value of 0.003.

13. In addition, Table 1 shows data extracted from Zee *et al.* (*Stroke* 37, 2007-2011, 2006, attached as Exhibit E) that reports additional association results for HapA in FLAP to MI (and stroke) in a cohort from the US (Physician Health Study). The frequency of HapA in this study was in observable excess in MI (and stroke) patients as compared to controls. Two distinct groups of controls were used (for MI and stroke). In Table 1, the association of HapA to MI is presented in a similar manner as our analysis, *i.e.* the control groups were joined to gain power and a chi- squared test with one degree of freedom was carried out. The one sided *P*-value is shown in Table 1. The relative risk of MI calculated for the HapA haplotype of Zee *et al.* is consistent with our findings.

14. The haplotype analysis was done using the program NEMO (Gretarsdottir *et al. Nat Genet* 35:131-138, 2003; Exhibit F). NEMO handles missing genotypes and uncertainty with phase through a likelihood procedure, using the expectation-maximization algorithm as a computational tool to estimate haplotype frequencies. For the at-risk haplotypes we calculated the relative risk (RR) assuming a multiplicative model (Falk and Rubinstein, *Ann Hum Genet* 51 (Pt 3):227-233, 1987;Terwilliger and Ott, *Hum Hered* 42:337-346, 1992, Exhibit G) in which the risk of the two alleles of haplotypes a person carries multiplies. Since we tested only one haplotype, which has previously been shown to confer risk of MI, the reported *P*-values are one sided. The cohort adjustments were performed using the model of Mantel and Haenszel, where each cohort is allowed to have different control haplotype frequencies, but the relative risk is assumed to be the same across cohorts. We extended the standard Mantel-Haenszel test in our NEMO program to take into account

the incomplete information on haplotype counts (Helgadottir *et al. Nat Genet* 38:68-74, 2006; Exhibit H).

15. Overall, the replication studies performed and reported to date, together with the metaanalysis data presented herein, substantiates the involvement of variants within the FLAP gene, in particular HapA, with MI and stroke.

16. I further declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both (18 U.S.C. § 1001), and may jeopardize the validity of the application or any patent issuing thereon.

Dated: 22.01.2007 Anna Helgadottir

Anna Helgadottir, M.D.